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THE EFFECT OF ZYMOSAN AND ENDOTOXIN TREATMENT ON EXPERIMENTAL COXSACKIE B VIRUS INFECTION

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ABSTRACT

Cold acclimatized and nonacclimatized adult albino mice were given varying doses of zymosan or endotoxin subcutaneously 48 hours prior to challenge with 20 X 10^4 plaque forming units of Coxsackie B-1 virus intraperitoneally. Neither agent was effective in increasing host resistance and, in some instances, appeared to enhance the disease process.

PUBLICATION REVIEW

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THE EFFECT OF ZYMOSAN AND ENDOTOXIN TREATMENT ON EXPERIMENTAL COXSACKIE B VIRUS INFECTION*

SECTION 1. INTRODUCTION

In a previously reported experiment (Marcus et al, 1963), mice were treated with zymosan or E. coli endotoxin prior to challenge with Coxsackie B-1 virus. The treatment doses and route of administration were those determined to be optimal for protection against Klebsiella pneumoniae challenge. The results indicated that endotoxin appeared to be detrimental to survival while zymosan may have offered some protection in the mice that were acutely cold exposed following challenge. The extent of protection afforded by either agent in the acclimatized animal was decreased and approximately of the same magnitude.

This report is a study of further investigation into the role of nonspecific immunity against experimentally induced viral disease.

SECTION 2. SUMMARY

Adult albino mice were treated with varying doses of zymosan or endotoxin subcutaneously prior to challenge with Coxsackie B-1 virus intraperitoneally. Neither agent was effective in protection and in some instances appeared to enhance the disease process. Animal groups tested included cold acclimatized, non-cold acclimatized and room temperature controls.

SECTION 3. MATERIAL AND METHODS

Coxsackie B-1 virus was obtained from the Department of Microbiology, University of Utah. The details for propagating and assaying the virus were previously reported (Marcus et al, 1963). Briefly, the virus was propagated in monkey kidney tissue culture and assayed according to the plaque technique of Dulbecco and Vogt (1954).

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*This research was conducted in accordance with the "Principles of Laboratory Animal Care" of the National Society for Medical Research.

Adult albino mice (Mus musculus) obtained from local sources were used in a random fashion (with no regard for sex) and the average weight of the animals at the initiation of the experiments was 21 to 22 gm.

The animals were exposed to 2° C either as nonacclimatized or acclimatized animals. Acclimatized animals were cold exposed for 45 to 50 days before challenge. Animals exposed to the cold were kept in groups of 10 in cages containing water and food ad libitum and with sawdust bedding just adequate to cover the cage bottom. Control animals were kept at 21° C. The temperatures of the rooms did not vary more than ± 1.5° C during the experimental period as monitored by calibrated temperature recording instruments.

Zymosan (lot OB298, Fleischmann Laboratories, Stamford, Conn.) was prepared by evenly suspending the insoluble carbohydrate complex in 10 volumes of 0.12 M NaCl. This suspension was placed in a boiling water bath for 60 minutes. Following this, the preparation was centrifuged for 30 minutes at 2180 g in a refrigerated International PR-2 Centrifuge. The supernatant fluid was discarded and the residue was resuspended in barbital buffer pH 7.4 to the desired concentration (Pillemer et al, 1956). The route of injection was subcutaneously in the nuchal region in a volume of 0.1 ml. The animals received 1, 4.5, 9 or 18 mg 48 hours prior to challenge.

Bacto lipopolysaccharide (Difco lot 0923, E. coli 055:B5) was carefully weighed out and suspended in pyrogen-free 0.15 M NaCl to the desired concentration. The material was given subcutaneously in the nuchal region in a volume of 0.1 ml. Each mouse received 1, 10 or 100 μg 48 hours prior to challenge.

The virus challenge was given intraperitoneally in a volume of 0.1 ml. The challenge dose was calculated on the basis of plaque forming units (PFU).

SECTION 4. RESULTS

It is seen in Table I that treatment with different doses of zymosan or endotoxin did not yield any protective effect against Coxsackie B-1 virus challenge in mice that were either acclimatized or nonacclimatized to 2° C. There is no significant protective effect from these agents. Further, endotoxin treatment in acclimatized mice appears to be slightly detrimental to survival under the experimental conditions. The results obtained with the control groups are very similar to those previously reported (Marcus et al, 1963); that is, the virus caused little mortality in the 21° or 2° C acclimatized mice, but exerted maximal disease in mice that were nonacclimatized to 2° C.

TABLE I

MORTALITY RATIOS AT 14 DAYS OF MICE TREATED WITH ZYMOSAN OR ENDOTOXIN AND CHALLENGED WITH COXSACKIE B-1 VIRUS (20×10^4 PFU) INTRAPERITONEALLY

ANIMAL GROUP	TREATMENT							
	None	Zymosan (mg)				Endotoxin (μg)		
		1	4.5	9	18	1	10	100
21° C	1/20	0/20	2/20	1/20	2/20	0/20	0/20	0/20
Non-acclimatized Mice 2° C	17/20	15/20	12/20	12/20	11/20	20/20	15/20	14/20
Acclimatized Mice 2° C	2/20	6/20	7/20	4/20	4/20	8/20	7/20	7/20

SECTION 5. DISCUSSION

The effects on bacterial disease of nonspecific immunizing agents such as those employed in the present study have been reported by other investigators, e. g., Landy (1956), Kiser et al (1956), Pillemer (1956), Braude and Siemienski (1961), and Springer et al (1961). The above references are selected to indicate that their observations are representative of the majority of the workers; that is, within certain doses and times of administration, one can either protect the host or enhance the bacterial disease process by injection of endotoxins or zymosan. These workers have not reported any extensive studies with respect to the effect of zymosan or endotoxin on viral disease processes.

Recently Nemes and Hilleman (1962) reported that Westphal's lipid A, which has endotoxic activity, enhanced nonspecific host resistance to the neurotoxicity of neurotropic influenza and herpes simplex viruses. In addition, this substance was active against influenza A and encephalomyocarditis viruses, but was inactive against Lansing type II poliovirus and Coxsackie B-3 virus. To our knowledge this is one of the first reports in the literature concerned with nonspecific resistance to viral diseases.

What is the role of zymosan and endotoxin in the pathogenesis of experimental viral disease? Since it has been shown that the properdin system will neutralize Newcastle disease virus (NDV) (Ginsberg and Wedgwood, 1956), and it also has been reported that normal fresh human, guinea pig, rabbit and mouse sera contain a heat-labile component that neutralizes influenza A, influenza B and mumps virus (Ginsberg and Horsfall, 1949), one would expect that the properdin system may be operative among the mechanisms of resistance to viral disease. However, the properdin system requires all four complement components for activity (Pillemer, 1956); yet the mouse has been shown to have little of at least two of the C' components (Rice and Crowson, 1950) and has been shown to be devoid of bactericidal activity (Marcus et al, 1954). Also, it is unlikely that the properdin system is contributing significantly to the defenses of the mouse (Miya et al, 1960).

The mechanism of action of the lipid A and endotoxin in inducing resistance to viruses is unknown, but Nemes and Hilleman (1962) suggest that enhanced functional capacity of the reticuloendothelial system is a factor. This is supported by the work of Biozzi et al (1955). In addition, endotoxin induces measurable alteration of the metabolism of macrophages and this may play a role in increased resistance to viral disease (Whitby et al, 1961). Gyi, Donaldson and Marcus (1955) and Perkins, Marcus, Gyi and Miya (1958) have shown that endotoxin in amounts of 1 μg twice daily for seven days will significantly enhance the intracellular digestive activities of mouse peritoneal macrophages.

The role of macrophages in influenza viral disease has been investigated by Boand et al (1957) who reported that the virus could be phagocytized in vitro and that the rate of phagocytosis was markedly enhanced in the presence of immune serum and by leucocytes from "immune" animals. These results were confirmed recently by Inglot and Davenport (1962).

In view of the paucity of information related to nonspecific mechanisms of defense against viral diseases, the results obtained in the present investigation are not surprising and serve as a baseline study for future experiments with regard to the role that macrophages play in host resistance to viral diseases. The role of cellular defense mechanisms in host resistance needs further investigation and should be extended into the field of virology.

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